Deposit structure and efficacy of pesticide application. 2: *Trichoplusia ni* control on cabbage with fipronil

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Abstract: Pesticide deposits have a spatial structure having elements of size, number per area and toxicant per deposit. To investigate the relative contributions of these elements to the efficacy of the deposit structure, we developed a bioassay using the cabbage looper (*Trichoplusia ni*), cabbage, and a soluble concentrate formulation of fipronil [(±)-5-amino-1-(2,6-dichloro-α,α,α-trifluoro-*p*-tolyl)-4-trifluoromethylsulfinylpyrazole-3-carbonitrile]. The bioassay manipulated deposit structure by changing the number, toxicant concentration of the solution, and size of droplets used in creating deposits. The bioassay methodology was developed as an extension from standard industrial mixture experimental designs. Results from the bioassay led to the following conclusions: (1) Deposit structure plays a major role in toxicant efficacy. (2) The effect of droplet size is roughly equal to the effect of concentration, while both these factors may have a greater effect than droplet number. (3) The interactions between the factors of deposit size, deposit number, and concentration are more important than any single component. (4) Uniform coverage is not the most efficacious deposit structure if one is forced to limit application rates, and field persistence. (5) Uniform deposit structures have less variability in their biological effect than do more heterogeneous structures–though the relationship is not linear. These bioassay data corroborate the predictions of an earlier paper.

Keywords: *Trichoplusia ni*; cabbage looper; mixture design; pesticide application; deposit structure; fipronil

1 INTRODUCTION

Previously we had identified a problem in the literature dealing with optimal droplet size.[1] There were problems in correlating laboratory and field results. Laboratory results showed that toxicants applied in smaller droplets were more effective than when they were applied in larger droplets. However, in the field, experiments with different application equipment provided conflicting evidence on the relationship between small droplet sizes and efficacy. This discrepancy was explained by redesigning the laboratory experimental methodology. Deposit size became one element of deposit structure. The other elements in deposit structure were number of deposits, the toxicant per deposit, and the total dose. With total dose held constant, any change in one of the other three resulted in a change in one or both of the remaining variables. Thus the factorial experimental design used in previous laboratory experiments is invalid because the 'independent' variables of size, number, and concentration are not independent. Consequently, we used a mixture design to examine the effects of deposit structure.[2]

A computer simulation (the Pesticide Dose Simulator [PDS] model) was used to investigate the consequences of deposit structure on mortality and damage.[1] Our theoretical investigations led to several conclusions, including the counter-intuitive insight that mortality and damage may not be equivalent measures of crop protection. It emerged from model predictions that deposit pattern influenced both percentage mortality and life span. Thus, delayed but high mortality could still result in serious damage, and reduced but early mortality result in superior crop protection. These predictions were obtained using a model originally designed to describe the dose-transfer process of *Bacillus thuringiensis* Berl (Bt) against *Plutella xylostella* (L) (Diamondback moth) on cabbage. While the model was originally designed around biological data, our experiment has taken the model in an unexpected direction which requires some additional biological validation.

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Treatment	Size (μm)	AI concentration (g litre^{-1})	Number	Mortality (%)	Standard Deviation
1	160	0.589	1800	37.5	0.236
2	397	0.300	232	21.4	0.184
3	988	0.300	15	8.9	0.135
4	2437	0.300	1	23.2	0.122
5	397	69.583	1	17.9	0.170
6	983	4.569	1	21.4	0.130
7	160	1059.723[a]	1	26.8	0.179
8	160	70.650	15	35.7	0.254
9	160	4.568	232	67.9	0.251
10	200	0.300	1800	28.6	0.210
11	179	0.420	1800	33.9	0.036
12	395	4.516	16	69.6	0.229

[a] This concentration was achieved by using pure formulation. X-77 was not added to this treatment because this would have diluted the level of AI.

Table 1. Treatment list for all experiments, along with percentage mortality by treatment averaged over all four replicates

A bioassay using *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) feeding on cabbage treated with fipronil [(±)-5-amino-1-(2,6-dichloro-α,α,α-trifluoro-*p*-tolyl)-4-trifluoromethylsulfinylpyrazole-3-carbonitrile] was used to generate data for a response surface analysis using the method developed for analyzing industrial mixture data. Deposit size, number and concentration were used to create different mixtures (or blends) of deposit structure, all with the same total dose applied to the entire leaf segment. While this methodology is applicable to studying the effects of deposit structure for all pesticides, our results are most pertinent to mobile chewing insects that are being controlled with an insecticide that has only minor sublethal effects and works primarily by ingestion.

2 METHODOLOGY

We have focused on deposit structure as a key element in the dose transfer process because it is the interface between pesticide application and the target organism. While deposit structure can be measured over spatial scales from micrometers to kilometers, we have selected a range from 100 μm to 10 cm. This scale is appropriate because it is the scale at which individual insects, mites, and fungi encounter pesticides. At this scale, deposit structure is the distribution of pesticide on the target surface. It is measured as the arrangement of different numbers of deposits of different sizes on the target surface, along with the dose per deposit. These features combine to produce a total dose per unit area. For a description of how to manipulate the data to enable the use of a mixture model for analyzing the effects of deposit structure, readers are referred to Ebert *et al.*[1]

Table 1 shows the range of values used in our experiments, while Fig 1 shows the arrangement of the values for this experimental design and illustrates how to interpret the graphs. For reference, Fig 1 also shows the recommended concentration for field application (dotted area) and the approximate range in volume median diameter (VMD) one might expect from atomizing these solutions through a flat fan nozzle at 40 psi (trapezoidal region bounded by dark lines). Special adjuvants or nozzles are required to get outside these bounds.

Cabbage (*Brassica oleracea* L cv *capitata*: cultivar All Season) was grown in the greenhouse in 53 × 26 × 5 cm black plastic flats half filled with Wooster Silt Loam. Peters Professional® water-soluble 20–20–20 (with micronutrients) was used for fertilizer. Plants were watered every day and fertilized twice per week. Cabbage plants used for experiments were four to eight weeks old.

Eggs of *T ni* were shipped to LPCAT (Laboratory for Pest Control Application Technology) from Abbott Laboratories (1401 N Sheridan Road, North Chicago, IL 60064), stored in a growth chamber at 10 °C, and hatched as needed – though eggs did not remain viable longer than 14 days. Larvae were reared on cabbage to the second instar in batches of several hundred.

Solutions of a 170 g litre^{-1} soluble concentrate formulation of fipronil (from Rhone-Poulenc Ag Company, PO Box 12014, TW Alexander Drive, Research Triangle Park, N C 27709) were made the afternoon before use. All solutions were diluted with distilled water containing 1 ml litre^{-1} X-77 (a nonionic spreader activator made by Loveland Industries, PO. Box, Greeley, CO 80632-1289, and containing 90% isopropanol, alkylarylpolyoxyethylene glycols, and free fatty acids, with 10% 'inert' ingredients.). This was added to enhance droplet retention on the waxy cabbage leaf surface. Unpublished LPCAT results have indicated no toxic effect from X-77 on *T ni* larvae at concentrations 10 times that used in these experiments.

Polystyrene Petri dishes (10 cm diameter × 1.5 cm high; Fisher Scientific 1600 W Glenlake Ave, Itasca, IL 60143) had a 9-cm diameter cellulose filter paper disk (Qualitative grade: Fisher Scientific) placed in the bottom. Disks were wetted with 1 ml of distilled water. This was sufficient water to reduce desiccation damage of the leaf disk, but not enough to drown larvae weakened by exposure to the insecticide.

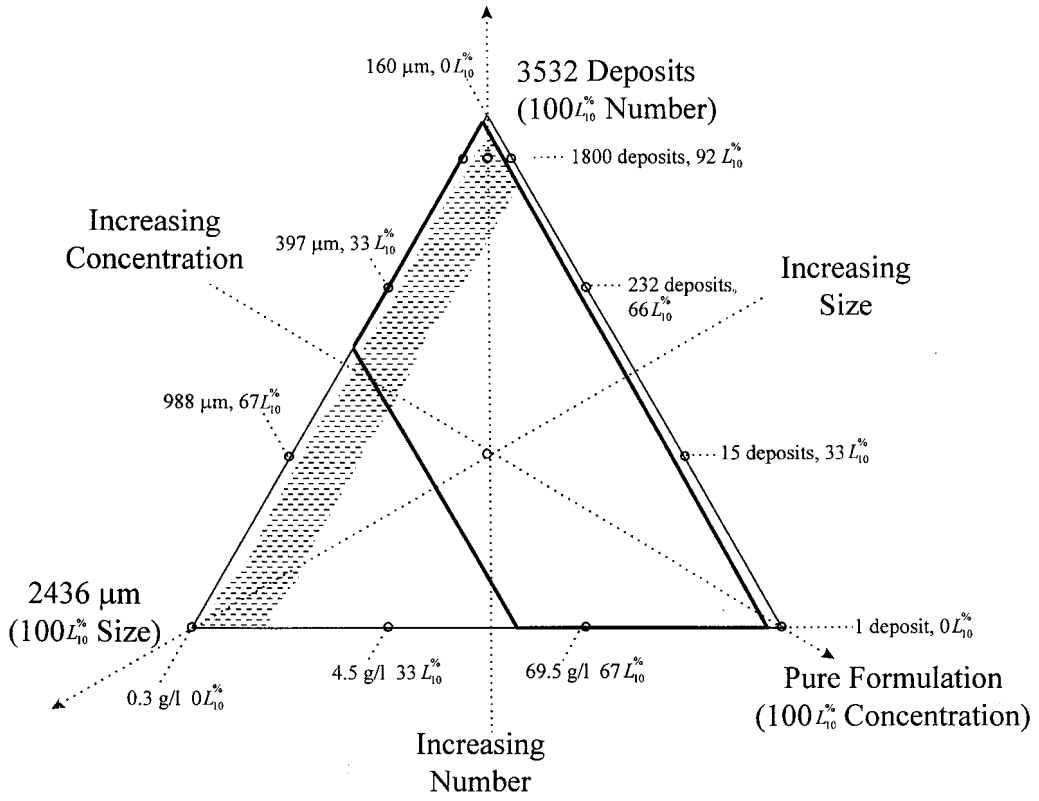


Figure 1. Interpreting the mixture design response surface graphs.

Leaf disks 4 cm in diameter were cut from cabbage leaves, avoiding the main veins in the leaves. Leaf disks were treated with 431.8 ng AI per disk using the LPCAT droplet generator (a piezoelectric pulse-driven droplet generator).[3] This dose should have been sufficient to kill all these larvae, but after 96 h most were large enough to consume the entire dose with little or no effect. The treatment list in Table 1 was the goal of the application. Prior to application, droplet size was checked by trapping droplets in Corning fluid and measuring their diameter with an ocular micrometer in a stereomicroscope which was calibrated using a stage micrometer consisting of 1 mm in 100 divisions. Sometimes the droplet generator did not produce the desired droplet size, and minor adjustments to the treatment list were made by changing the number of droplets to account for the change in droplet size. This gives rise to the variation in the position of data points in the different graphs.

Droplets were allowed to dry prior to placing the leaf disk and one *T ni* larva in a Petri dish. One larva per dish eliminated problems of cannibalism and ensured that there was no 'competition' for deposits from other larvae. After placing larvae in Petri dishes, additional larvae were removed from the colony and weighed. Larvae were chosen which looked similar to those used for the experiment. Table 2 shows the number of larvae sampled, and their average weight. Petri dishes were placed in an incubator (Precision Scientific model 808). Temperature was a constant 22.5 °C, with 0:24 h L:D. Larvae were exposed to light and ambient temperatures for up to 30 min each day while mortality was assessed. Mortality was assessed every 24 h for 96 h. Larvae were considered dead only after all movement had ceased, and contact with a camel-hair brush failed to elicit movement. After assessing mortality, an additional 300 μl of distilled water was added to Petri dishes to compensate for desiccation.

Leaf disks from Petri dishes with dead larvae (or all larvae at 96 h) were photographed using a black and white Kodak megaplus digital camera. Image Pro+ version 3.0 (Media Cybernetics, 8484 Georgia Ave, Silver Spring, MD 20910) was used to measure area eaten. In some cases leaf area remaining was measured and area eaten found by subtraction, with the assumption that the leaf area was 12.57 cm^2. Either method resulted in some level of error because the leaf disk would expand and warp over the 96-day period. Distortion of the image caused by warping of the leaf

Table 2. Average biomass of test insects and the average percentage mortality by replicate

Replicate	Number of larvae	Biomass per larva	Mortality (%)		
			Min	Max	Average
1	564	0.001389	0	50	27.4
2	258	0.001248	0	93	30.4
3	392	0.001347	0	71	36.9
4	84	0.002023	7	93	36.3

Table 3. Regression equations for percentage mortality and area eaten

Mortality run		Size (A)	Concentration (B)	Number (C)	AB	AC	BC	ABC	AB(A-B)	AC(A-C)	BC(B-C)	Adjusted R^2	Significance level	Lack of fit
1	Coefficients	−0.574	−0.185	0.260	−0.929	−5.036	−1.265	17.722		10.561		0.800	<.001	0.143
	Standard error	0.153	0.149	0.178	0.701	0.767	0.813	5.329		1.712				
	Significance				0.20	<0.00	0.14	<0.00		<0.00				
2	Coefficients	−0.674	−0.773	−0.460	−1.100	−4.521	0.320	25.141				0.146	0.192	0.321
	Standard error	0.406	0.406	0.334	1.902	1.929	1.931	14.031						
	Significance				0.57	0.03	0.87	0.09						
3	Coefficients	−1.517	−1.296	−1.362	1.324	0.044	4.748	16.538		−7.056	−6.988	0.443	0.023	0.157
	Standard error	0.370	0.370	0.466	1.711	1.887	1.890	12.577		4.048	4.037			
	Significance				0.45	0.82	0.02	0.21		0.10	0.10			
4	Coefficients	−0.510	−0.602	−1.258	−1.972	0.242	−0.814	30.191	6.137	−10.754	1.158	0.399	0.047	0.223
	Standard error	0.320	0.320	0.462	1.440	1.718	3.324	16.774	3.209	3.500	4.650			
	Significance				0.19	0.89	0.81	0.09	0.08	0.01	0.81			
Average	Coefficients	−0.805	−0.800	−0.481	−0.459	−2.874	1.039	19.889				0.230	<0.001	0.284
	Standard error	0.184	0.183	0.158	0.859	0.897	0.904	6.456						
	Significance				0.59	<0.00	0.25	<0.00						
Area														
1	Coefficients	0.512	0.272	0.458	0.943	0.283	0.246	−4.348				0.090	0.002	0.098
	Standard error	0.065	0.065	0.057	0.304	0.321	0.325	2.307						
	Significance				<0.00	0.38	0.45	0.06						
2	Coefficients	0.408	0.372	0.248	0.475	0.477	0.245	−7.451				0.210	<0.001	0.316
	Standard error	0.041	0.041	0.034	0.194	0.196	0.197	1.429						
	Significance				0.02	0.02	0.21	<0.00						
3	Coefficients	0.488	0.411	0.358	0.117	0.541	−0.863	−5.196		1.077	1.466	0.340	<0.001	0.583
	Standard error	0.053	0.053	0.067	0.245	0.270	0.271	1.798		0.579	0.579			
	Significance				0.63	0.05	<0.00	<0.00		0.07	0.01			
4	Coefficients	0.373	0.374	0.319	−0.360	−0.050	0.235	−6.133		−6.133		0.160	<0.001	0.011
	Standard error	0.049	0.047	0.063	0.225	0.256	0.503	2.544		0.533				
	Significance				0.11	0.84	0.64	0.02		0.01				
Average	Coefficients	0.386	0.213	0.005	0.150	0.363	−0.170	−8.821				0.215	<0.001	0.502
	Standard error	0.089	0.089	0.077	0.418	0.437	0.441	3.145						
	Significance				0.72	0.41	0.70	0.01						

disk was reduced by placing a glass plate over it prior to photography.

The data consist of observations from four replicates of the 12 treatments (Table 1, Fig 1). For each of the 12 treatments, 14 larvae were tested (a total of 168 larvae). The first replicate was started on 4 March 1998, and repeated on 5 May 1998, 11 May 1998, and 10 July 1998 (a grand total of 672 larvae). For each date, an additional treatment (14 larvae) was added as a control. The control leaf disks were not treated. Except for a single larva in the control for 10 July 1998, no mortality was observed in the controls. The data are not corrected for control mortality. Percentage mortality was calculated using results from seven of the 14 Petri dishes for each treatment. This provided two estimates of percentage mortality for each treatment: the first seven dishes and last seven dishes. Thus there are 24 observations for percentage mortality for each date. In calculating the average result, area eaten was averaged using the same grouping used to get percentage mortality. The estimation of standard deviation for the area eaten was arrived at using the same arrangement of data.

Analysis was done in the experimental design module of Statistica release 5.1H (StatSoft. Inc, 2300 East 14th Street, Tulsa, OK 74104). All dependent variables were log transformed prior to analysis. All independent variables had already been log transformed in designing the experiment.

3 RESULTS

Percentage mortality is the most commonly used measure of product efficacy. Over all of our experiments and treatments, the percentage mortality was 32.7%. Table 2 lists the breakdown by experiment, and shows that mortality ranges from a low of 0% to a high of 90% within an experiment. This range is brought about by changes in deposit structure. Deposit structure was poorest for treatment 3 (9% mortality) and best for treatment 12 (70% mortality) (Table 1).

Plate 1 shows the percentage mortality response surface graphs for each replicate. The center of each graph is close to the maximum response. There is an area of reduced efficacy at the lowest concentration and lowest droplet number. Table 3 shows the regression equations for the replicates analyzed individually, and for all four replicates. Increasing droplet size decreases percentage mortality. Increasing concentration decreases percentage mortality. Increasing droplet number decreases efficacy. However, the interaction terms between these factors are stronger than the effects of these terms alone. Dominant among the interaction terms is the cubic interaction of Size*Concentration*Number. This term accounts for the central peak in the regression. The only significant quadratic term is the Size*Number interaction.

Plate 2 shows the area eaten response surface graphs for each replicate. Like the percentage mortality

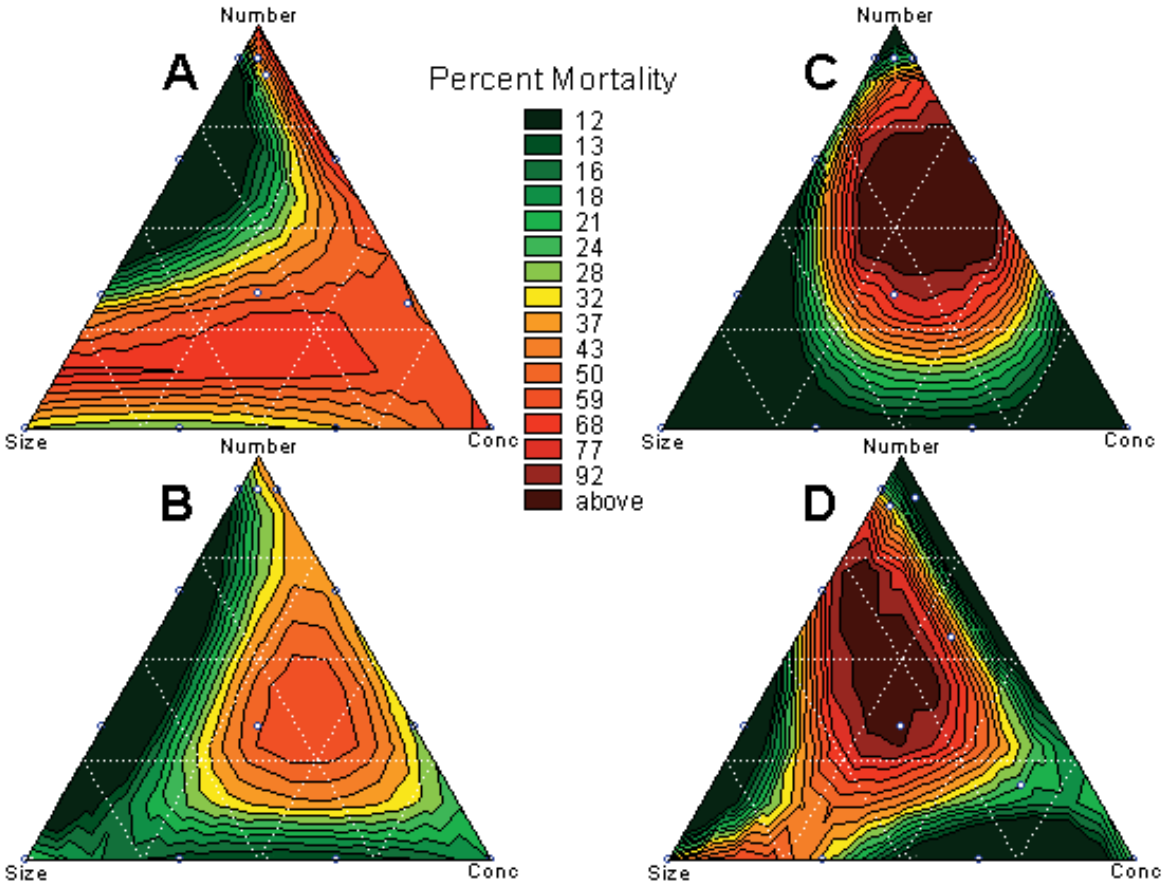


Plate 1. Percent mortality influenced by deposit structure. Letters correspond to dates: A is 4 March 1998, B is 5 May 1998, C is 11 May 1998 and D is 10 July 1998.

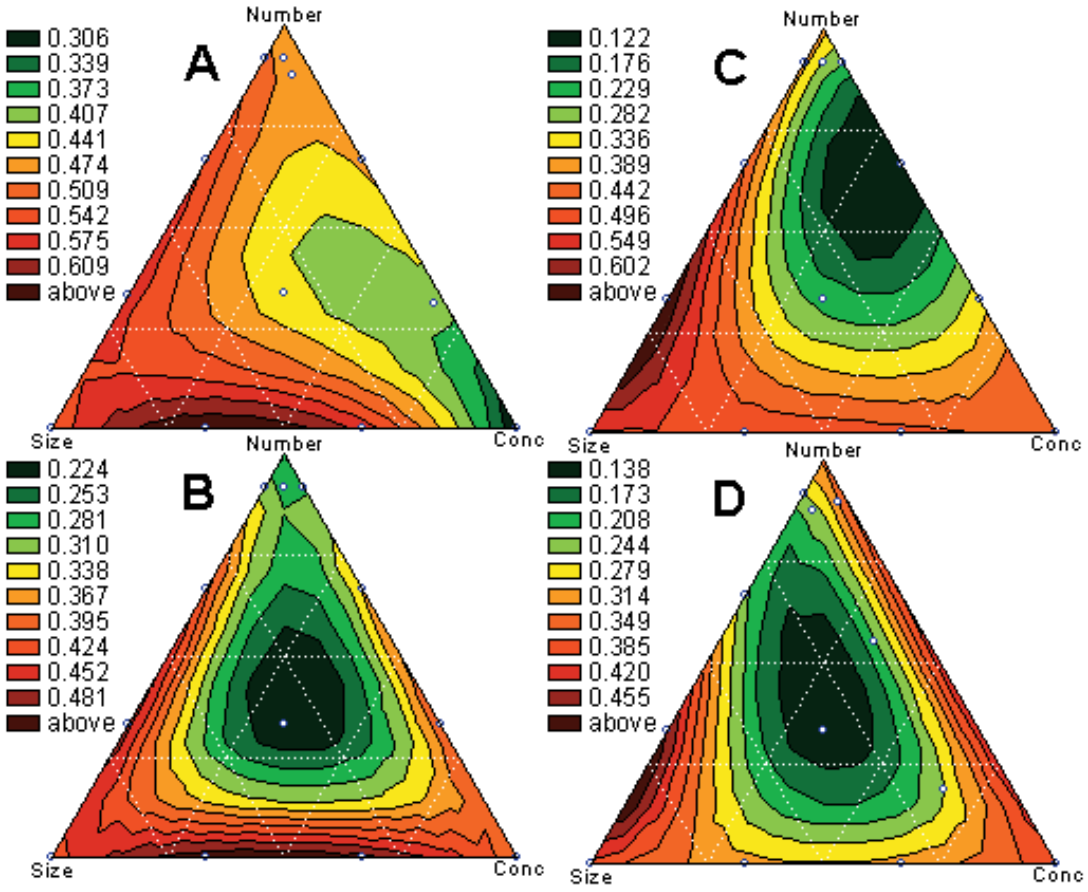
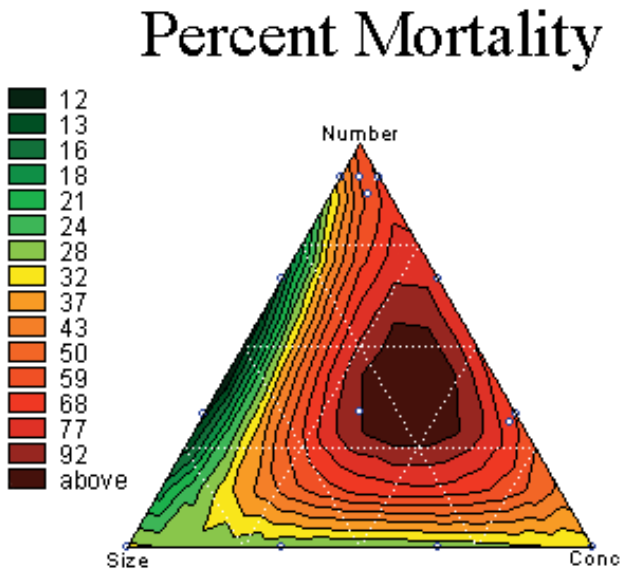
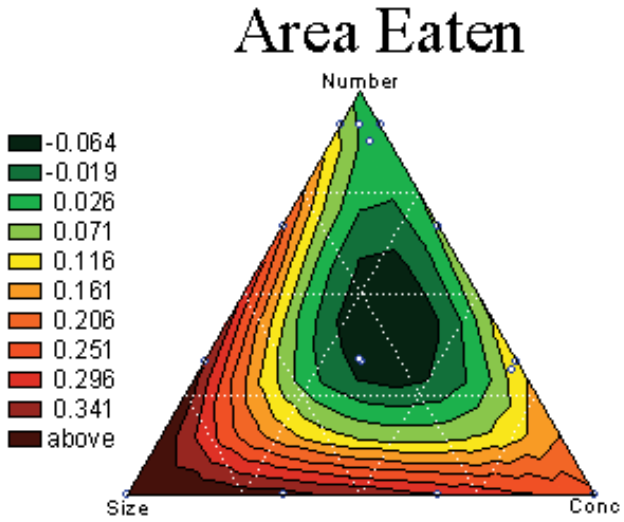


Plate 2. Area eaten influenced by deposit structure. Letters correspond to dates: A is 4 March 1998, B is 5 May 1998, C is 11 May 1998 and D is 10 July 1998.





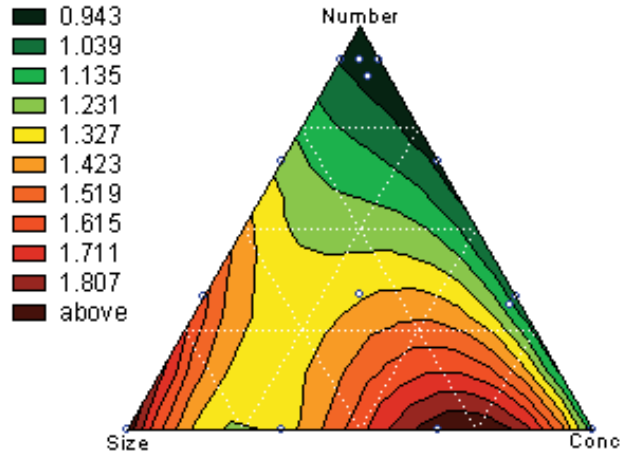


Plate 3. Average response in percentage mortality, area eaten, and variability in area eaten as influenced by deposit structure.

graphs, these also show a region of greatest efficacy in the center of the response surface. Table 3 shows the fitted equations. Larger droplets are less effective at protecting the crop. Higher concentrations are less effective at protecting the crop. More droplets are less effective at protecting the crop. However, once again, it is the interaction among these three factors which is most important in determining application efficacy. Specially, it is the cubic interaction of Size*Concentration*Number which dominates the system under these conditions.

Plate 3 shows the results from the analysis when all four replicates are modeled as a single experiment. The results from this analysis are similar to that from analysis of individual replicates for percentage mortality and area eaten (Table 3, Plate 3). In addition, there is a graph showing the standard deviation for area eaten. It shows low standard deviation at high droplet numbers, and more variable results as the number of droplets declines.

4 DISCUSSION

While we have provided the regression equations for each model, we would like to emphasize the patterns in Plates 1, 2 and 3 rather than numerical results. This is because these specific results were derived under laboratory conditions. There has been some interest in questions like 'what is the effect of droplet size.' Our results provide an answer to that question. However, our results are subject to the usual list of caveats necessary when extrapolating laboratory data to the 'real' world. These experiments were deliberately designed to be as simple as possible, both to aid an understanding of the application process and to provide a starting point in developing the complex statistical models required for more realistic experiments.

In the introduction we suggested that better coverage does not always result in greater efficacy. These results illustrate how this might happen. With a uniform deposit, insects can take longer to die, providing time for further growth and consumption of leaf tissue. In addition, AI in the deposit may decay and the plant may grow, both of which decrease the dose per unit area. Thus 'better' coverage provides poorer control when the total dose is limited (average mortality about 33%). We emphasize that this conclusion is the same for both the computer simulation[1] and the bioassay.

We stated in the introduction that small deposits appear to be more effective in the laboratory, but application methodologies applying small droplets do not consistently produce better results.[4] Because the dominant feature in these particular experiments was a hill (or valley), factorial experiments which start at the edges and end near the middle will show one effect. Experiments which go in the opposite direction will show the opposite effect, and those which move along a contour interval will show no effect at all. Thus, our results are consistent with all previously published work, even where the previous results appear contradictory. More importantly, these results are consistent with the model results previously published.[1] Compare the percentage mortality graphs with the equivalent from the PDS model (see previous paper,[1] Fig 5, at time = 4000 min) where the average mortality was about 36.1%. Clearly, both the computer model and the bioassay produce a central region of higher mortality when the average mortality is about 33%.

One difference between the bioassay results and the computer simulation is the elevated mortality at high droplet numbers in the bioassay results. We hypothesize that this is due to coalescence of the droplets in forming deposits. The computer simulation has some coalescence when droplets directly impact each other. However, in the bioassay this problem is greater because in the process of deposit formation, highly waxy areas will promote redistribution of droplets into less waxy areas. Thus, while 1800 droplets may produce 1200 deposits in the computer simulation, they may only produce 600 deposits in the bioassay. Hence the apparent effect of high droplet number is reduced in the bioassay. Thus, we need some method for analyzing the spatial arrangement of deposits which would bridge the information gap between the deposit structure from a field application and these laboratory results. This is one of the current research areas at LPCAT.

The correlation coefficient between percentage mortality and damage is 0.55 in these bioassays. This is lower than the 0.76 observed in the computer simulation. However, while the low correlation between mortality and damage in the bioassay would usually be attributed to biological variation, the computer simulation would suggest that some of this is an emergent property of the system and not just a function of the random noise present in any biological system.

Aspects of these results impact all of application technology. Figure 1 showed the experimental area targeted by a typical application of this product (the intersection of the hatched area and the trapezoidal boundary). This is a small fraction of the possible ways to apply this product, as can be seen from comparing the size of the area targeted by the typical application versus the entire area bounded by the graph. The targeted area also does not usually contain the region of maximum efficacy, as shown in the plates.

By changing our view of the application process we have identified deposit structures which should make it possible to reduce application rates to a fraction of their current levels and retain efficacy if new application methodologies are developed. Focusing exclusively on creating uniform deposit structures (as produced by large numbers of very small droplets) restricts the scope of experimentation and innovation to a small portion of the potential ways target organisms can encounter toxicants. This approach to examining deposit structure applies to all pest control

agents where deposit quality influences efficacy (eg fungicides,[5] herbicides[6–8]). We have little doubt that improved targeting of AI can result in both reduced rates and increased efficacy. However, we must be clear about our objectives (protection versus mortality) and cognizant of the effects of spatial scale (whole field versus individual insects).

ACKNOWLEDGEMENTS

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